PRELIMINARY REPORT ON THE EARLY HISTORY OF THE EGG AND EMBRYO OF CERTAIN HYDROIDS.

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Within the last few years some of the hydroids (*Pennaria, Clava leptostyla, Eudendrium, Tubularia crocea*) have been described as differing widely in their processes of maturation, fertilization, and early cleavage from what we have come to think of as typical. Other members of the same group (*Tubularia mesembryantheum, Clava squamata, Hydra, Gonothyrea, Æquorea, Tiara, Gonionemus*) have been described as perfectly typical. That certain of the hydroids should conform to the type and others not, seemed improbable. The fact of the low organization of the group which is used by Hargitt to account for such variation seemed hardly sufficient. On this account at the suggestion of Professors Morgan and Wilson, I undertook the reëxamination of two of the forms, *Pennaria* and *Clava leptostyla,* which varied most from the type, to discover if possible the relation between the aberrations described in these forms and the usual type. The results on *Pennaria* were worked out in the winter of 1907 at the Zoölogical Laboratory of Columbia University, on material collected at Woods Hole during the previous summer. Those on *Clava* were obtained in the summer and autumn of 1908.

A brief review of Hargitt's work on *Pennaria* and *Clava leptostyla* will be necessary to recall the points which I wished to clear up. In both these forms, according to his results, the processes of maturation and fertilization of the egg are very obscure and incapable of demonstration. The germinal vesicle just before the time that maturation should take place moves to the periphery of the egg where it loses its staining capacity, the nuclear membrane breaks down, and the nuclear substance becomes diffused throughout the egg, where it is no longer recognizable, due probably to some chemical or physical change in the egg. The

maturation and fertilization processes were supposed to take place while the chromatin is in this unstaining condition, the sperm having also lost its staining capacity after entering the egg. The first evidence of nuclei in the egg after the disappearance of the germinal vesicle was found in the appearance of "nuclear nests" or groups of small vesicles scattered throughout the egg, several such nests usually occurring in an unsegmented egg. In *Pennaria* not less than four such nests appear simultaneously indicating four centers of nuclear reconstruction. In *Clava,* as I understand the description, this condition of several nuclear groups occurs occasionally in an unsegmented egg. More often, however, eggs were found already segmented with a single resting nucleus in each cell which I take it were believed to arise as described for *Pennaria.* This reappearance Hargitt thinks is explicable on the ground that after fertilization the chemical or physical conditions again change so that the chromatin once more responds to stains. The chromatic material that was scattered at the time the nucleus disappeared collects again, forms vesicles which especially in *Pennaria* occur in groups or nests, each nest finally fusing into a single nucleus. Thus a syncytium arises without mitosis and with no apparent evidence of maturation or fertilization having taken place in the egg. In *Pennaria* after these nuclear groups are formed, nuclear proliferation is by mitosis. In *Clava leptostyla,* however, up to the sixteen-cell stage Hargitt describes nuclear proliferation by amitosis, later cleavages being mitotic.

The points in question accordingly are: (1) The nature of maturation and fertilization processes, (2) the formation of nuclei *de novo,* and (3) the *rôle* of amitosis and mitosis in early cleavages.

Pennaria and *Clava leptostyla* were preserved at Woods Hole where Hargitt obtained his material. Also the same killing fluids and stains were used so that the question of method is eliminated. Material was killed every hour of the day and night and every half hour in the early morning hours which proved to be the most important period.

I found in both *Pennaria* and *Clava leptostyla* that in material killed between the hours of 4 and 6 A. M., it was possible to

demonstrate the maturation processes and that they take place with the utmost exactness and in the typical manner, as reference to the figures will show. Fig. 1 shows the germinal vesicle of an egg of *Pennaria* in which the inner wall is breaking down, the nucleolus passing into the cytoplasm where it is lost, while the chromatin is grouping itself around the periphery of the nucleus to form the chromosomes. Figs. 2 and 11 are nearly corresponding stages of the first polar spindle in *Pennaria* and *Clava leptostyla* respectively. In both forms the chromosomes are evidently bipartite and the number is determined to be one half the somatic number. In the figure of *Pennaria* (which is in the late prophase) the spindle has not yet swung around into position. A comparison of Figs. 3 and 12 shows again practically identical conditions of the second polar spindle in the two forms. The first polar body lies outside the egg, the second polar spindle is in the late anaphase. I have found numerous intermediate stages. Figs. 4 and 13 show corresponding stages of the reconstructed egg nucleus with the polar bodies lying outside the egg.

Figs. 5 and 6 give two stages in the fertilization of *Pennaria.* The two-germ nuclei lying side by side at the periphery of the egg later move toward the center of the egg where they form the fusion nucleus at the ends of which astral radiations appear. The origin of the first cleavage spindle is not determined. For lack of proper stages the fusion of the two-germ nuclei has not been demonstrated with certainty in *Clava leptostyla.*

From this point forward the two forms differ slightly. In *Pennaria,* after the first cleavage the two nuclei are reconstructed by the formation of chromosomal vesicles as shown in Figs. 7 and 8. For some reason, possibly the rapidity of nuclear divisions, the chromosomal vesicles often fail to fuse into a single nucleus but give rise to a "nuclear nest" which subsequently gives rise directly to the chromosomes of the following cleavage figure. Fig. 9 shows two such vesicles passing on to a spindle, one vesicle already broken up into the individual chromosomes, the other still in the vesicular stage. Fig. 10 shows an equatorial view of such a group of vesicles, some of the chromosomes already forming an equatorial plate.

A shortening of the resting stage between the nuclear divisions,

it seems to me, might account for Hargitt's interpretation of the "nuclear nests." The rapidity of nuclear division is accompanied by slow cytoplasmic division so that the former constantly outruns the latter, the result being that an unsegmented egg often contains several such groups of vesicles or "nuclear nests."

In *Clava* the cytoplasmic cleavage does not lag so far behind the nuclear division, but in fact keeps pace with it. For this reason it was possible as shown by Figs. 14, 15, 16, 17, 18 and 19 to demonstrate the first cleavage spindle, and the successive passage of the two-cell stage into the four-cell, eight-cell and sixteen-cell stages. The nuclear reconstruction takes place here again by the formation of chromosomal vesicles; but *Clava* differs from *Pennaria* in that, as a rule, the vesicles all fuse into a single nucleus between successive cleavages So far as I am able to tell as yet, it seems probable that the cleavage in *Clava* is fairly regular.

Summary.

In the two hydroids (*Pennaria* and *Clava leptostyla*) under question, the maturation and fertilization processes take place in a perfectly typical fashion and form no exception to the general rule in this regard.

The conclusion that the "nuclear nests" indicate the formation of nuclei *de novo* is shown to be untenable. The occurrence of these nests is explained by the conditions of nuclear reconstruction after cleavage, the chromosomal vesicles failing to fuse between successive divisions in *Pennaria* and the cytoplasmic division lagging behind nuclear division gives a syncytium with several nuclear groups.

Maturation and the early cleavages take place by means of mitosis and not amitosis. No evidence whatever of amitotic division was found.

My results regarding the maturation and fertilization phenomena make it very probable that Hargitt's failure to observe these stages was due simply to the fact that the eggs were not obtained at the right time of day. In eggs collected at the proper time (4–6 A. M.) there is no difficulty in proving the typical stages of maturation and fertilization.

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EXPLANATION OF PLATE I.

Pennaria. All drawings × 1,300.

FIG. 1. Early prophase of germinal vesicle of egg of *Pennaria;* the inner nuclear wall is breaking down; the nucleolus passing into the cytoplasm; the chromosomes forming.

FIG. 2. Later prophase of first polar spindle of egg of *Pennaria;* some of the chromosomes bipartite; some quadripartite. Reconstructed from two consecutive sections.

FIG. 3. Anaphase of second polar spindle of egg of *Pennaria.*

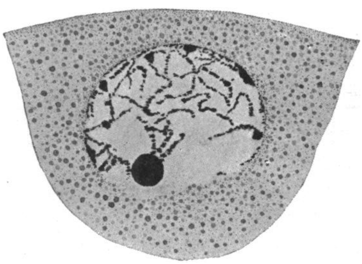
FIG. 4. Female germ nucleus and polar bodies in egg of *Pennaria;* chromatin is in fine reticulum.

FIG. 5. Fertilization of *Pennaria* egg; male and female germ nuclei at the periphery of the egg and of equal size. Reconstructed from three consecutive sections.

FIG. 6. Egg of *Pennaria* showing the fusion nucleus with astral radiations at either end of the nucleus.

FIG. 7. Telophase of 1st cleavage of egg of *Pennaria;* nuclear reconstruction by the formation of chromosomal vesicles.

FIG. 8. "Nuclear nests" formed by the partial fusion of the chromosomal vesicles lying at the ends of the spindle (probably second or third cleavage). *Pennaria.*



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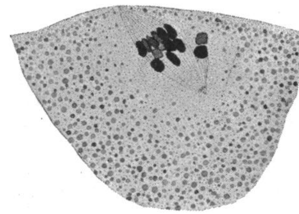

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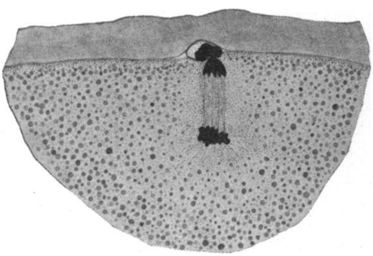

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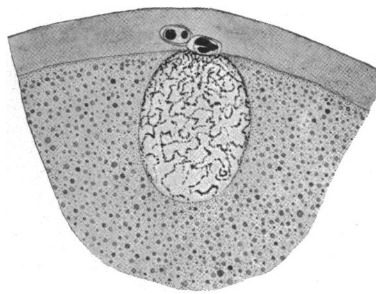

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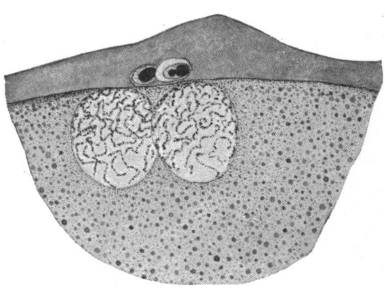

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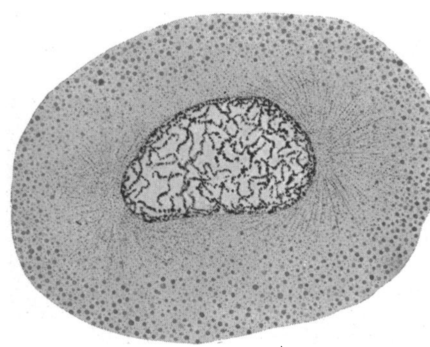

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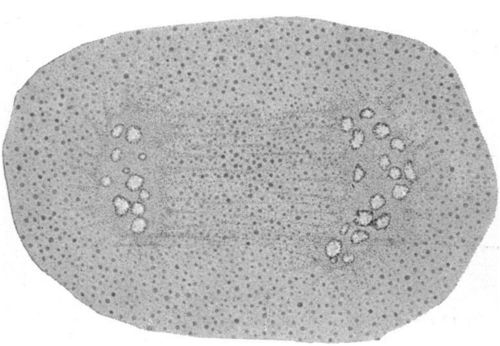

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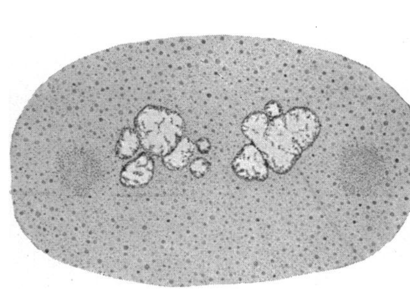

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Explanation of Plate II.

Pennaria. Figs. 9 and 10, × 1,300.

Clava leptostyla. Figs. 11 to 13, × 1,300; Fig. 14, × 350.

Fig. 9. Third or fourth cleavage spindle with "nuclear nest" passing on to it to form the equatorial plate, one vesicle broken up into chromosomes. *Pennaria.*

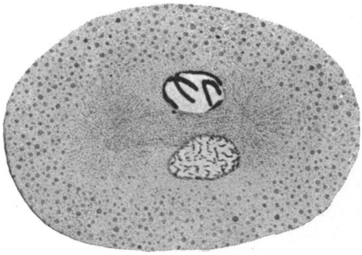
Fig. 10. Polar view of a cleavage spindle showing a "nuclear nest" as it is breaking up to form the equatorial plate, slightly later than the above, some of the chromosomes already free in the cytoplasm. *Pennaria.*

Fig. 11. Metaphase of first polar spindle of egg of *Clava leptostyla.*

Fig. 12. Anaphase of the second polar spindle of egg of *Clava leptostyla.*

Fig. 13. Female germ nucleus and polar bodies of egg of *Clava leptostyla.*

Fig. 14. Egg of *Clava leptostyla* showing the first cleavage spindle.



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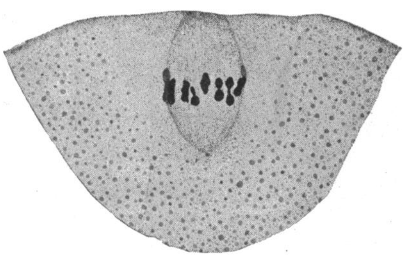

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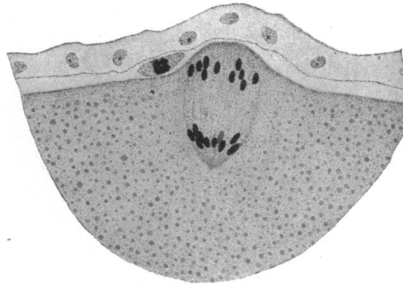

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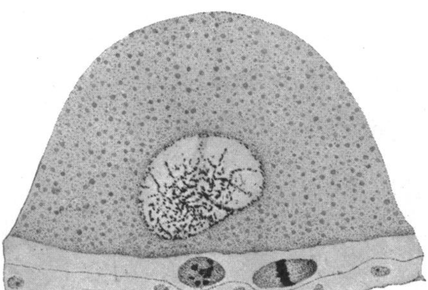

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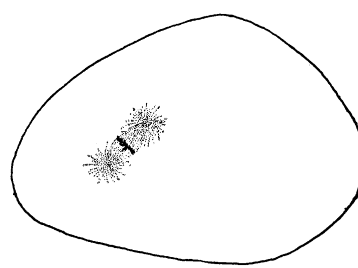

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Explanation of Plate III.

Clava leptostyla. Figs. 15 to 19, × 350.

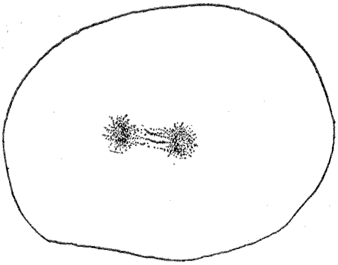
FIG. 15. Same, showing reconstruction of daughter nuclei by chromosomal vesicles.

FIG. 16. Two-cell stage of *Clava leptostyla* passing into the four-cell stage, second cleavage spindles showing.

FIG. 17. Four-cell stage of *Clava leptostyla* with resting nuclei.

FIG. 18. Four-cell stage of *Clava* passing into the eight-cell stage, spindles showing in two cells.

FIG. 19. Lateral view of eight-cell stage of *Clava* (five cells showing in section), some of the cells with spindles, some with resting nuclei.



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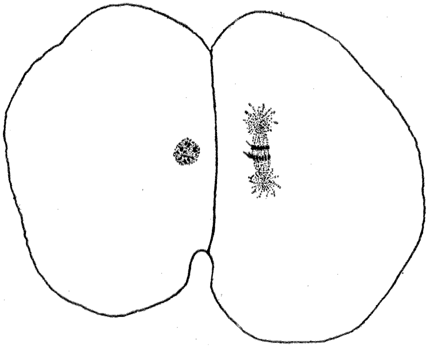

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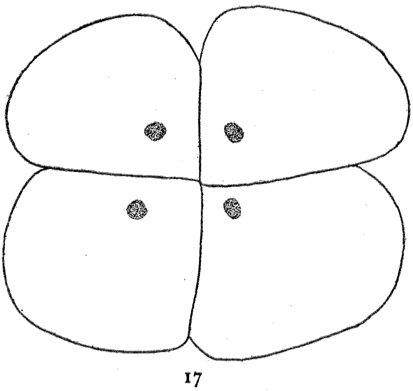

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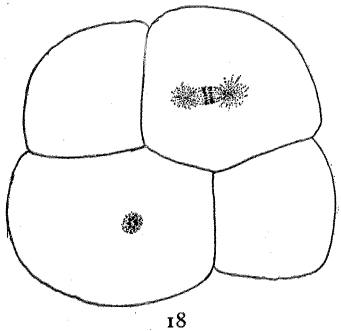

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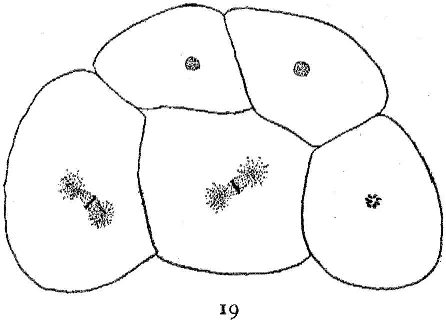

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